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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	OCT 23	The Derwent World Patents Index suite of databases on STN has been enhanced and reloaded
NEWS 4	OCT 30	CHEMLIST enhanced with new search and display field
NEWS 5	NOV 03	JAPIO enhanced with IPC 8 features and functionality
NEWS 6	NOV 10	CA/CAplus F-Term thesaurus enhanced
NEWS 7	NOV 10	STN Express with Discover! free maintenance release Version 8.01c now available
NEWS 8	NOV 20	CA/CAplus to MARPAT accession number crossover limit increased to 50,000
NEWS 9	DEC 01	CAS REGISTRY updated with new ambiguity codes
NEWS 10	DEC 11	CAS REGISTRY chemical nomenclature enhanced
NEWS 11	DEC 14	WPIDS/WPINDEX/WPIX manual codes updated
NEWS 12	DEC 14	GBFULL and FRFULL enhanced with IPC 8 features and functionality
NEWS 13	DEC 18	CA/CAplus pre-1967 chemical substance index entries enhanced with preparation role
NEWS 14	DEC 18	CA/CAplus patent kind codes updated
NEWS 15	DEC 18	MARPAT to CA/CAplus accession number crossover limit increased to 50,000
NEWS 16	DEC 18	MEDLINE updated in preparation for 2007 reload
NEWS 17	DEC 27	CA/CAplus enhanced with more pre-1907 records
NEWS 18	JAN 08	CHEMLIST enhanced with New Zealand Inventory of Chemicals
NEWS 19	JAN 16	CA/CAplus Company Name Thesaurus enhanced and reloaded
NEWS 20	JAN 16	IPC version 2007.01 thesaurus available on STN
NEWS 21	JAN 16	WPIDS/WPINDEX/WPIX enhanced with IPC 8 reclassification data
NEWS 22	JAN 22	CA/CAplus updated with revised CAS roles
NEWS 23	JAN 22	CA/CAplus enhanced with patent applications from India
NEWS 24	JAN 29	PHAR reloaded with new search and display fields
NEWS 25	JAN 29	CAS Registry Number crossover limit increased to 300,000 in multiple databases
NEWS 26	FEB 13	CASREACT coverage to be extended
NEWS 27	Feb 15	PATDPASPC enhanced with Drug Approval numbers
NEWS 28	Feb 15	RUSSIAPAT enhanced with pre-1994 records
NEWS 29	Feb 23	KOREAPAT enhanced with IPC 8 features and functionality
NEWS 30	Feb 26	MEDLINE reloaded with enhancements
NEWS 31	Feb 26	EMBASE enhanced with Clinical Trial Number field
NEWS 32	Feb 26	TOXCENTER enhanced with reloaded MEDLINE
NEWS 33	Feb 26	IFICDB/IFIPAT/IFIUDB reloaded with enhancements
NEWS 34	Feb 26	CAS Registry Number crossover limit increased from 10,000 to 300,000 in multiple databases

NEWS EXPRESS NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006.

NEWS HOURS STN Operating Hours Plus Help Desk Availability

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 NEWS LOGIN     Welcome Banner and News Items
 NEWS IPC8      For general information regarding STN implementation of IPC 8
 NEWS X25       X.25 communication option no longer available

Enter NEWS followed by the item number or name to see news on that
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FILE 'HOME' ENTERED AT 11:53:30 ON 13 MAR 2007

=> ile medlinef
ILE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILE 'MEDLINE' ENTERED AT 11:53:51 ON 13 MAR 2007

 FILE LAST UPDATED: 10 Mar 2007 (20070310/UP).  FILE COVERS 1950 TO DATE.

  All regular MEDLINE updates from November 15 to December 16 have been
  added to MEDLINE, along with 2007 Medical Subject Headings (MeSH(R))
  and 2007 tree numbers.

  The annual reload will be available in early 2007.

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

=> phage and holin
PHAGE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s phage and holin
         28623 PHAGE
           121 HOLIN
L1          87 PHAGE AND HOLIN

=> s l1 and pna
          4711 PNA
L2             0 L1 AND PNA

=> s l1 and antisense
         25390 ANTISENSE
L3             0 L1 AND ANTISENSE

=> s l1 and ribozyme
          3072 RIBOZYME
L4             0 L1 AND RIBOZYME
```

=> s l1 and transport
286978 TRANSPORT
L5 2 L1 AND TRANSPORT

=> d ab 1-2

L5 ANSWER 1 OF 2 MEDLINE on STN
AB The Lyz endolysin of bacteriophage P1 was found to cause lysis of the host without a holin. Induction of a plasmid-cloned lyz resulted in lysis, and the lytic event could be triggered prematurely by treatments that dissipate the proton-motive force. Instead of requiring a holin, export was mediated by an N-terminal transmembrane domain (TMD) and required host sec function. Exported Lyz of identical SDS/PAGE mobility was found in both the membrane and periplasmic compartments, indicating that periplasmic Lyz was not generated by the proteolytic cleavage of the membrane-associated form. In gene fusion experiments, the Lyz TMD directed PhoA to both the membrane and periplasmic compartments, whereas the TMD of the integral membrane protein FtsI restricts Lyz to the membrane. Thus, the N-terminal domain of Lyz is both necessary and sufficient not only for export of this endolysin to the membrane but also for its release into the periplasm. The unusual N-terminal domain, rich in residues that are weakly hydrophobic, thus functions as a signal-arrest-release sequence, which first acts as a normal signal-arrest domain to direct the endolysin to the periplasm in membrane-tethered form and then allows it to be released as a soluble active enzyme in the periplasm. Examination of the protein sequences of related bacteriophage endolysins suggests that the presence of an N-terminal signal-arrest-release sequence is not unique to Lyz. These observations are discussed in relation to the role of holins in the control of host lysis by bacteriophage encoding a secretory endolysin.

L5 ANSWER 2 OF 2 MEDLINE on STN
AB Listeria monocytogenes bacteriophages A118, A500 and A511 are members of three distinct phage groups with characteristic host ranges. Their endolysin (ply) genes were cloned and expressed in Escherichia coli as demonstrated by the conferred lytic phenotype when colonies of recombinant cells were overlaid with a lawn of Listeria cells. The nucleotide sequences of the cloned DNA fragments were determined and the individual enzymes (PLY118, 30.8 kDa; PLY500, 33.4 kDa; PLY511, 36.5 kDa) were shown to have varying degrees of homology within their N-terminal or C-terminal domains. Transcriptional analysis revealed them to be 'late' genes with transcription beginning 15-20 min post-infection. The enzymes were overexpressed and partially purified and their individual specificities examined. When applied exogenously, the lysins induced rapid lysis of Listeria strains from all species but generally did not affect other bacteria. Using hydrolysis of purified listerial cell walls, PLY511 was characterized as an N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) and shows homology in its N-terminal domain to other enzymes of this type. In contrast, PLY118 and PLY500 were shown to represent a new class of cell wall lytic enzymes which cleave between the L-alanine and D-glutamate residues of listerial peptidoglycan; these were designated as L-alanoyl-D-glutamate peptidases. These two enzymes share homology in the N-terminal domain which we propose determines hydrolytic specificity. Highly conserved holin (hol) gene sequences are present upstream of ply118 and ply500. They encode proteins of structural similarity to the product of phage lambda gene S, and are predicted to be membrane proteins which form pores to allow access of the lysins to their peptidoglycan substrates. This arrangement of conserved holin genes with downstream lysin genes among the siphoviral lysis cassettes explains why the cytoplasmic endolysins alone are not lethal, since they require a specific transport function across the cell membrane.

=> s l1 and antibacterial

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        24774 ANTIBACTERIAL
L6          1 L1 AND ANTIBACTERIAL

=> d ab

L6   ANSWER 1 OF 1     MEDLINE on STN
AB   To characterize the enzymatic activity and antibacterial
     activity of endolysin encoded by a Bacillus amyloliquefaciens
     phage, the open reading frame encoding endolysin was amplified by
     PCR and cloned into the expression plasmid pET21d(+). The resultant
     plasmid was used to transform Escherichia coli JM109(DE3). Production of
     endolysin in the cytosol facilitated cell lysis without coproduction of
     holin, which is considered to degrade or alter the cytoplasmic
     membrane. The phage endolysin was overexpressed and purified.
     Although the specific activity of the purified phage endolysin
     towards lyophilized Micrococcus luteus cells was 1/11 of the activity of
     chicken egg white lysozymes, the endolysin showed stronger
     antibacterial activity towards E. coli W3110, E. coli JM109(DE3)
     and Pseudomonas aeruginosa PAO1 than chicken egg white lysozymes. The
     antibacterial activity of the endolysin towards these three
     bacterial strains was marked when EDTA was added to the endolysin
     solution.


=> s l1 and cell wall
       2103676 CELL
        156557 WALL
         25927 CELL WALL
                 (CELL(W)WALL)
L7          19 L1 AND CELL WALL

=> d ab 1-19

L7   ANSWER 1 OF 19     MEDLINE on STN
AB   The fate of phage-infected bacteria is determined by the
     holin, a small membrane protein that triggers to disrupt the
     membrane at a programmed time, allowing a lysozyme to attack the
     cell wall. S(21)68, the holin of
     phage 21, has two transmembrane domains (TMDs) with a predicted
     N-in, C-in topology. Surprisingly, TMD1 of S(21)68 was found to be
     dispensable for function, to behave as a SAR ("signal-anchor-release")
     domain in exiting the membrane to the periplasm, and to engage in
     homotypic interactions in the soluble phase. The departure of TMD1 from
     the bilayer coincides with the lethal triggering of the holin
     and is accelerated by membrane depolarization. Basic residues added at
     the N terminus of S(21)68 prevent the escape of TMD1 to the periplasm and
     block hole formation by TMD2. Lysis thus depends on dynamic topology, in
     that removal of the inhibitory TMD1 from the bilayer frees TMD2 for
     programmed formation of lethal membrane lesions.

L7   ANSWER 2 OF 19     MEDLINE on STN
AB   Double-stranded DNA phages of both Gram-positive and Gram-negative
     bacteria typically use a holin-endolysin system to achieve lysis
     of their host. In this study, the lysis genes of Staphylococcus aureus
     phage P68 were characterized. P68 gene lys16 was shown to encode
     a cell-wall-degrading enzyme, which causes cell lysis
     when externally added to clinical isolates of S. aureus. Another gene,
     hol15, was identified embedded in the -1 reading frame at the 3' end of
     lys16. The deduced Hol15 protein has three putative transmembrane
     domains, and thus resembles class I holins. An additional candidate
     holin gene, hol12, was found downstream of the endolysin gene
     lys16 based on two predicted transmembrane domains of the encoded protein,
     which is a typical trait of class II holins. The synthesis of either
     Hol12 or Hol15 resulted in growth retardation of Escherichia coli, and
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both hol15 and hol12 were able to complement a phage lambda Sam mutation. The hol15 gene has a dual start motif beginning with the codons Met1-Lys2-Met3.... Evidence is presented that the hol15 gene encodes a lysis inhibitor (anti-holin) and a lysis effector (actual holin). As depolarization of the membrane converted the anti-holin to a functional holin, these studies suggested that hol15 functions as a typical dual start motif class I holin . The unusual arrangement of the P68 lysis genes is discussed.

L7 ANSWER 3 OF 19 MEDLINE on STN

AB An open reading frame encoding an 88 amino acid protein was present downstream of the previously characterized endolysin of Streptomyces aureofaciens phage micro1/6. Structural analysis of its sequence revealed features characteristic for holin. This open reading frame encoding the putative holin was amplified by polymerase chain reaction and cloned into the expression vector pET-21d(+). Synthesis of the holin-like protein resulted in bacterial cell death but not lysis. The holmicro1/6 gene was able to complement the defective lambda S allele in the nonsuppressing Escherichia coli HB101 strain to produce phage progeny, This fact suggests that the proteins encoded by both phage genes have analogous function, i.e. the streptomycete holin induces nonspecific lesions in the cytoplasmic membrane, through which the lambda endolysin gains an access to its substrate, the cell wall. The concomitant expression of both S. aureofaciens holmicro 1/6 and lambda endolysin in E. coli resulted in abrupt cell lysis. This result provided further evidence that the product of holmicro 1/6 gene is a holin .

L7 ANSWER 4 OF 19 MEDLINE on STN

AB An open reading frame homologous to the genes encoding several cell-wall hydrolyzing enzymes was identified on the genome of actinophage mu 1/6. This open reading frame encoding the putative endolysin was amplified by polymerase chain reaction and cloned into the expression vector pET-21a. This gene consisted of 1182 bp encoding a 393 amino acid polypeptide with a molar mass of 42.1 kDa. The gene product was overexpressed in Escherichia coli, and then the lytic enzyme was purified by a two-step chromatographic procedure. When applied exogenously, the endolysin of phage mu 1/6 was active against all tested Streptomyces strains but did not affect other bacteria. The amino acid sequence showed a high homology with a putative amidase of the Streptomyces phase phi C31. Downstream of the endolysin gene, an open reading frame encoding an 88 amino acid protein was identified. Structural analysis of its sequence revealed features characteristics for holin.

L7 ANSWER 5 OF 19 MEDLINE on STN

AB AIMS: To evaluate the ability of a filamentous phage encoding lethal proteins to kill bacteria without host-cell lysis. METHODS AND RESULTS: Bacterial survival was determined after infection of a growing Escherichia coli culture with phage M13 encoding either the restriction endonuclease BglII gene or modified phage lambda S holin genes. The genetically engineered phage exerted a high killing efficiency while leaving the cells structurally intact. When compared with a lytic phage, the release of endotoxin was minimized after infection with the genetically modified phages. CONCLUSIONS: Genetically engineered phage can be used for efficient killing, concomitantly minimizing endotoxin release. SIGNIFICANCE AND IMPACT OF THE STUDY: This feasibility study provides a possible strategy for the use of genetically engineered phage as bactericidal agents by optimizing the advantages and minimizing potential risks such as release of pyrogenic cell wall components.

L7 ANSWER 6 OF 19 MEDLINE on STN

AB Bacteriophages must destroy the bacterial cell wall to lyse their host and release their progeny into the environment. There are at least two distinct mechanisms by which phages destroy the cell wall. Bacteriophages with large genomes use a holin -endolysin system, while bacteriophages with small genomes encode a single lysis protein. Three unrelated single protein lysis systems are known and these proteins will be the focus of the review. Recent results indicate that at least two of these proteins inhibit cell wall synthesis and are thus the phage analogs of antibiotics like penicillin.

L7 ANSWER 7 OF 19 MEDLINE on STN

AB Clostridium perfringens commonly occurs in food and feed, can produce an enterotoxin frequently implicated in food-borne disease, and has a substantial negative impact on the poultry industry. As a step towards new approaches for control of this organism, we investigated the cell wall lysis system of C. perfringens bacteriophage phi3626, whose dual lysis gene cassette consists of a holin gene and an endolysin gene. Hol3626 has two membrane-spanning domains (MSDs) and is a group II holin. A positively charged beta turn between the two MSDs suggests that both the amino terminus and the carboxy terminus of Hol3626 might be located outside the cell membrane, a very unusual holin topology. Holin function was experimentally demonstrated by using the ability of the holin to complement a deletion of the heterologous phage lambda S holin in lambdadeltaSthf. The endolysin gene ply3626 was cloned in Escherichia coli. However, protein synthesis occurred only when bacteria were supplemented with rare tRNA(Arg) and tRNA(Ile) genes. Formation of inclusion bodies could be avoided by drastically lowering the expression level. Amino-terminal modification by a six-histidine tag did not affect enzyme activity and enabled purification by metal chelate affinity chromatography. Ply3626 has an N-terminal amidase domain and a unique C-terminal portion, which might be responsible for the specific lytic range of the enzyme. All 48 tested strains of C. perfringens were sensitive to the murein hydrolase, whereas other clostridia and bacteria belonging to other genera were generally not affected. This highly specific activity towards C. perfringens might be useful for novel biocontrol measures in food, feed, and complex microbial communities.

L7 ANSWER 8 OF 19 MEDLINE on STN

AB Holins are integral membrane proteins that control the access of phage-encoded muralytic enzymes, or endolysins, to the cell wall by the sudden formation of an uncharacterized homo-oligomeric lesion, or hole, in the membrane, at a precisely defined time. The timing of lambda-infected cell lysis depends solely on the 107 codon S gene, which encodes two proteins, S105 and S107, which are the holin and holin inhibitor, respectively. Here we report the results of biochemical and genetic studies on the interaction between the holin and the holin inhibitor. A unique cysteine at position 51, in the middle of the second transmembrane domain, is shown to cause the formation of disulfide-linked dimers during detergent membrane extraction. Forced oxidation of membranes containing S molecules also results in the formation of covalently linked dimers. This technique is used to demonstrate efficient dimeric interactions between S105 and S107. These results, coupled with the previous finding that the timing of lysis depends on the excess of the amount of S105 over S107, suggest a model in which the inhibitor functions by titrating out the effector in a stoichiometric fashion. This provides a basis for understanding two evolutionary advantages provided by the inhibitor system, in which the production of the inhibitor not only causes a delay in the timing of lysis, allowing the assembly of more virions, but also increases effective hole formation after triggering.

L7 ANSWER 9 OF 19 MEDLINE on STN

AB The genes encoding the host cell wall-lytic proteins were searched in the genome DNA of phage PL-1 active against Lactobacillus casei ATCC 27092 by comparing the amino acid sequences with those of others using a computer software of the DDBJ data base. The gene regions found were cloned into E. coli by inserting PCR-amplified DNA fragments into the EcoRI site of pUC 19, and the nucleotide sequences were determined. One of the ORFs (hol) consisted of 270 bp encoding 90 amino acids. The hol product (holin) possessed a putative secretion signal, two putative transmembrane helices, and a highly charged C-terminus. Another ORF (lys) consisted of 1050 bp encoding an N-acetylmuramoyl-L-alanine amidase of 350 amino acids. The gene lys was expressed in E. coli using pCALn expression vector, and the purified gene product hydrolysed the amide linkage in the peptidoglycans of L. casei. The amino acid sequence of PL-1 amidase showed a high homology to those of Lactococcus lactis phage r1t and Listeria monocytogenes phage A511. It was suggested that the N-terminal region was involved in enzyme activity and the C-terminal region in binding the enzyme to the cell wall substrate, respectively.

L7 ANSWER 10 OF 19 MEDLINE on STN

AB We have cloned, sequenced, and characterized the genes encoding the lytic system of the unique Staphylococcus aureus phage 187. The endolysin gene ply187 encodes a large cell wall-lytic enzyme (71.6 kDa). The catalytic site, responsible for the hydrolysis of staphylococcal peptidoglycan, was mapped to the N-terminal domain of the protein by the expression of defined ply187 domains. This enzymatically active N terminus showed convincing amino acid sequence homology to an N-acetylmuramoyl-L-alanine amidase, whereas the C-terminal part, whose function is unknown, revealed striking relatedness to major staphylococcal autolysins. An additional reading frame was identified entirely embedded out of frame (+1) within the 5' region of ply187 and was shown to encode a small, hydrophobic protein of holin-like function. The hol187 gene features a dual-start motif, possibly enabling the synthesis of two products of different lengths (57 and 55 amino acids, respectively). Overproduction of Hol187 in Escherichia coli resulted in growth retardation, leakiness of the cytoplasmic membrane, and loss of de novo ATP synthesis. Compared to other holins identified to date, Hol187 completely lacks the highly charged C terminus. The secondary structure of the polypeptide is predicted to consist of two small, antiparallel, hydrophobic, transmembrane helices. These are supposed to be essential for integration into the membrane, since site-specific introduction of negatively charged amino acids into the first transmembrane domain (V7D G8D) completely abolished the function of the Hol187 polypeptide. With antibodies raised against a synthetic 18-mer peptide representing a central part of the protein, it was possible to detect Hol187 in the cytoplasmic membrane of phage-infected S. aureus cells. An important indication that the protein actually functions as a holin in vivo was that the gene (but not the V7D G8D mutation) was able to complement a phage lambda Sam mutation in a nonsuppressing E. coli HB101 background. Plaque formation by lambdagt11::hol187 indicated that both phage genes have analogous functions. The data presented here indicate that a putative holin is encoded on a different reading frame within the enzymatically active domain of ply187 and that the holin is synthesized during the late stage of phage infection and found in the cytoplasmic membrane, where it causes membrane lesions which are thought to enable access of Ply187 to the peptidoglycan of phage -infected Staphylococcus cells.

L7 ANSWER 11 OF 19 MEDLINE on STN

AB Upon infecting populations of susceptible host cells, T-even bacteriophages maximize their yield by switching from lysis at about 25 to 35 min at 37 degrees C after infection by a single phage

particle to long-delayed lysis (lysis inhibition) under conditions of sequential infection occurring when free phages outnumber host cells. The timing of lysis depends upon gene t and upon one or more rapid-lysis (r) genes whose inactivation prevents lysis inhibition. t encodes a holin that mediates the movement of the T4 endolysin though the inner cell membrane to its target, the cell wall. The rI protein has been proposed to sense superinfection. Of the five reasonably well characterized r genes, only two, rI and rV, are clearly obligatory for lysis inhibition. We show here that rV mutations are alleles of t that probably render the t protein unable to respond to the lysis inhibition signal. The tr alleles cluster in the 5' third of t and produce a strong r phenotype, whereas conditional-lethal t alleles produce the classical t phenotype (inability to lyse) and other t alleles produce additional, still poorly understood phenotypes. tr mutations are dominant to t+, a result that suggests specific ways to probe T4 holin function.

L7 ANSWER 12 OF 19 MEDLINE on STN

AB Holins are small membrane proteins that, at a genetically programmed time in a bacteriophage infective cycle, allow bacteriolytic enzymes, or endolysins, to escape to the periplasm and to attack the cell wall. Most holins fall into two sequence classes, I and II, based on the number of potential transmembrane domains (three for class I and two for class II). The prototype class I holin gene, S lambda, has a dual start motif and encodes not only the effector holin, Slambda105, but also an inhibitor, Slambda107, with a Met-Lys ...extension at the terminus. The prototype class II holin gene of phage 21, S 21, begins with the motif Met-Lys-Ser-Met ..., and a potential RNA secondary structure overlaps the Shine-Dalgarno sequence. Here, we demonstrate that (i) two protein products are elaborated from S 21, S2171 and S2168; (ii) the shorter product is required for lysis; (iii) the longer product, S2171, inhibits S 21 function; and (iv) the Lys-2 residue is important for the inhibitor function. Moreover, the RNA stem-loop structure is involved in the downregulation of S2171 synthesis. However, our results suggest that, in S 21, different segments of the single consensus Shine-Dalgarno sequence serve the two translational starts. These results show that the dual start motifs of class II holin genes are functionally homologous to those of class I holin genes.

L7 ANSWER 13 OF 19 MEDLINE on STN

AB The lysis genes of the virulent Staphylococcus aureus bacteriophage Twort were cloned and their nucleotide sequences determined. The endolysin gene plyTW encodes a 53.3-kDa protein, whose catalytic site is located in the amino-terminal domain. An enzymatically active fragment (N-terminal 271 amino acids) was overexpressed in Escherichia coli and partially purified. The enzyme rapidly cleaves staphylococcal peptidoglycan, and was shown to act as N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28). Significant sequence homology to the specific cell wall targeting domain of lysostaphin was observed in a 101-amino acid C-terminal overlap. However, we found that the large C-terminal portion (63%, 295 aa) of PlyTW is not required for staphylolytic activity. Located upstream of and overlapping plyTW by 35 bp in a different reading frame (+1), we identified holTW, which starts with a single TTG triplet. The gene specifies a 185-amino acid (20.5 kDa) holin protein, which features two potential hydrophobic, antiparallel transmembrane domains, and a highly charged, acidic C-terminus. HolTW is the largest class II holin described to date. It can substitute for the defective allele in phase lambda S' amber mutants, both in trans from an expression plasmid, and from within gt11::holTW. The proposed function is the formation of unspecific membrane lesions to promote access of the endolysin to the bacterial peptidoglycan.

L7 ANSWER 14 OF 19 MEDLINE on STN

AB Lactobacillus plantarum phage phi gle has two consecutive cell lysis genes hol-lys (Oki et al., 1996b). In the present study, functional and structural properties of the hol protein (Hol) were characterized in Escherichia coli. Electron microscopic examinations showed that hol under plac in E. coli XL1-Blue injured the inner membrane to yield empty ghost cells with the bulk of the cell wall undisturbed. Northern blot analysis indicated that hol-lys genes under plac were co-transcribed, although the amount of hol transcript was larger than that of lys, ceasing via an apparently rho-independent terminator just downstream of hol. However, deletion and/or fusion experiments suggested that: (1) the N-terminal half of phi gle Hol composed of three putative transmembrane domains may be responsible for interaction with membrane; (2) the N-terminal end (five amino acids) seems nonessential; and (3) the C-terminal half containing charged amino acids appears to be involved in proper hol function. These results suggest that phi gle Hol is a member of the lambdoid holin family, but divergent in several properties from lambda holin.

L7 ANSWER 15 OF 19 MEDLINE on STN

AB We have characterized four families of pneumococcal phages with remarkable morphologic and physiological differences. Dp-1 and Cp-1 are lytic phages, whereas HB-3 and EJ-1 are temperate phages. Interestingly, Cp-1 and HB-3 have a terminal protein covalently linked to the 5' ends of their lineal DNAs. In the case of Dp-1, we have found that the choline residues of the teichoic acid were essential components of the phage receptors. We have also developed a transfection system using mature DNAs from Dp-4 and Cp-1. In the later case, the transfecting activity of the DNA was destroyed by treatment with proteolytic enzymes, a feature also shared by the genomes of several small Bacillus phages. DNA replication was investigated in the case of Dp-4 and Cp-1 phages. The terminal protein linked to Cp-1 DNA plays a key role in the peculiar mechanism of DNA replication that has been coined as protein-priming. Recently, the linear 19,345-bp double-stranded DNA of Cp-1 has been completely sequenced, several of its gene products have been analyzed, and a complete transcriptional map has been ellaborated. Most of the pneumococcal lysins exhibit an absolute dependence of the presence of choline in the cell wall substrate for activity, and phage lysis requires, as reported for other systems, the action of a second phage-encoded protein, the holin, which presumably forms some kind of lesion in the membrane. The two lytic gene cassettes, from EJ-1 and Cp-1 phages, have been cloned and expressed in heterologous and homologous systems. The finding that some lysogenic strains of Streptococcus pneumoniae harbor phage remnants has provided important clues on the interchanges between phage and bacteria and supports the view of the chimeric origin of phages.

L7 ANSWER 16 OF 19 MEDLINE on STN

AB The ply genes encoding the endolysin proteins from Bacillus cereus phages Bastille, TP21, and 12826 were identified, cloned, and sequenced. The endolysins could be overproduced in Escherichia coli (up to 20% of total cellular protein), and the recombinant proteins were purified by a two-step chromatographical procedure. All three enzymes induced rapid and specific lysis of viable cells of several Bacillus species, with highest activity on B. cereus and B. thuringiensis. Ply12 and Ply21 were experimentally shown to be N-acetylmuramoyl-L-alanine amidases (EC 3.5.1.28). No apparent holin genes were found adjacent to the ply genes. However, Ply21 may be endowed with a signal peptide which could play a role in timing of cell lysis by the cytoplasmic phage endolysin. The individual lytic enzymes (PlyBa, 41.1 kDa; Ply21, 29.5 kDa, Ply12, 27.7 kDa) show remarkable heterogeneity, i.e., their amino acid sequences reveal only little homology. The N-terminal part of Ply21 was found to be almost identical to the catalytic domains of a Bacillus sp. cell wall hydrolase (CwlSP) and an autolysin of B. subtilis (CwlA). The C terminus of PlyBa contains a 77-amino-acid

sequence repeat which is also homologous to the binding domain of CwlSP. Ply12 shows homology to the major autolysins from B. subtilis and E. coli. Comparison with database sequences indicated a modular organization of the phage lysis proteins where the enzymatic activity is located in the N-terminal region and the C-termini are responsible for specific recognition and binding of Bacillus peptidoglycan. We speculate that the close relationship of the phage enzymes and cell wall autolysins is based upon horizontal gene transfer among different Bacillus phages and their hosts.

L7 ANSWER 17 OF 19 MEDLINE on STN

AB The lysis genes of a Lactobacillus phage phi g1e were cloned, sequenced, and expressed in Escherichia coli. Nucleotide sequencing of a 3813-bp phi g1e DNA revealed five successive open reading frames (ORF), Rorf50, Rorf118, hol, and lys and Rorf175, in the same DNA strand. By comparative analysis of the DNA sequence, the putative hol product (holin) has an estimated molecular weight is 14.2 kDa, and contains two potential transmembrane helices and highly charged N- and C-termini, resembling predicted holins (which are thought to be a cytoplasmic membrane-disrupting protein) encoded by other phages such as mv1 from Lactobacillus bulgaricus, phi adh from Lactobacillus gasseri, as well as monocins from Listeria. On the other hand, the putative phi g1e lys product (lysin) of 48.4 kDa shows significant similarity with presumed muramidase, known as a cell wall peptidoglycandegrading enzyme, encoded by the Lactobacillus phage mv1 and phi adh, the Lactococcus lactis phage phi LC3, and the Streptococcus pneumoniae phages Cp-1, Cp-7 and Cp-9. When expressed in E. coli, the phi g1e lysin and/or holin decreased the cell turbidity significantly, suggesting that the phi g1e hol-lys system is involved in cytolytic process.

L7 ANSWER 18 OF 19 MEDLINE on STN

AB The holin function Ejh of the pneumococcal bacteriophage EJ-1 has been characterized. It shows structural features similar to, and functionally complemented, the prototype member of the holin family. In Escherichia coli and Pseudomonas putida the Ejh product caused cellular death, and changes in cell morphology could be accounted for by lesions in the cytoplasmic membrane. Expression of ejh resulted in the inhibition of growth in a variety of phylogenetically distant bacterial genera, suggesting a broad spectrum of action. Concomitant expression of the ejh and ejl (encodes a lysin) genes led to lysis of E. coli and P. putida cells. Remarkably, the Ejl lysin was able to attack murein from bacteria lacking choline in their sacculi, which suggests that pneumococcal lysins have a broader substrate specificity than previously assumed. Furthermore, the Ejh holin was able to trigger activity of the major pneumococcal autolysin cloned and expressed in E. coli, and this raised new questions about the regulation of this model autolysin. A new function for holins in systems where the phage lysin is supposed to be associated with the membrane is proposed.

L7 ANSWER 19 OF 19 MEDLINE on STN

AB Listeria monocytogenes bacteriophages A118, A500 and A511 are members of three distinct phage groups with characteristic host ranges. Their endolysin (ply) genes were cloned and expressed in Escherichia coli as demonstrated by the conferred lytic phenotype when colonies of recombinant cells were overlaid with a lawn of Listeria cells. The nucleotide sequences of the cloned DNA fragments were determined and the individual enzymes (PLY118, 30.8 kDa; PLY500, 33.4 kDa; PLY511, 36.5 kDa) were shown to have varying degrees of homology within their N-terminal or C-terminal domains. Transcriptional analysis revealed them to be 'late' genes with transcription beginning 15-20 min post-infection. The enzymes were overexpressed and partially purified and their individual specificities examined. When applied exogenously, the lysins induced rapid lysis of Listeria strains from all species but generally did not

affect other bacteria. Using hydrolysis of purified listerial cell walls, PLY511 was characterized as an N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) and shows homology in its N-terminal domain to other enzymes of this type. In contrast, PLY118 and PLY500 were shown to represent a new class of cell wall lytic enzymes which cleave between the L-alanine and D-glutamate residues of listerial peptidoglycan; these were designated as L-alanoyl-D-glutamate peptidases. These two enzymes share homology in the N-terminal domain which we propose determines hydrolytic specificity. Highly conserved holin (hol) gene sequences are present upstream of ply118 and ply500. They encode proteins of structural similarity to the product of phage lambda gene S, and are predicted to be membrane proteins which form pores to allow access of the lysins to their peptidoglycan substrates. This arrangement of conserved holin genes with downstream lysin genes among the siphoviral lysis cassettes explains why the cytoplasmic endolysins alone are not lethal, since they require a specific transport function across the cell membrane.

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=> FIL STNGUIDE

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
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